nature immunology

Review article

https://doi.org/10.1038/s41590-023-01551-9

Immunology of human fibrosis

Received: 16 April 2023

Accepted: 8 June 2023

Published online: 20 July 2023

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Fibrosis, defined by the excess deposition of structural and matricellular proteins in the extracellular space, underlies tissue dysfunction in multiple chronic diseases. Approved antifibrotics have proven modest in efficacy, and the immune compartment remains, for the most part, an untapped therapeutic opportunity. Recent single-cell analyses have interrogated human fibrotic tissues, including immune cells. These studies have revealed a conserved profile of scar-associated macrophages, which localize to the fibrotic niche and interact with mesenchymal cells that produce pathological extracellular matrix. Here we review recent advances in the understanding of the fibrotic microenvironment in human diseases, with a focus on immune cell profiles and functional immune–stromal interactions. We also discuss the key role of the immune system in mediating fibrosis regression and highlight avenues for future study to elucidate potential approaches to targeting inflammatory cells in fibrotic disorders.

Fibrosis is characterized by the deposition of collagen and other extracellular matrix (ECM) molecules, mostly by fibroblasts, and is a major cause of tissular dysfunction in chronic diseases. Because of its pathologic role in disorders of the liver, heart, kidney, and lung, among others, as much as 45% of mortality in the USA has been estimated to be attributable to fibrosis[1]. Indeed, in disorders such as chronic liver disease (CLD), the degree of organ fibrosis is the best-known predictor of adverse long-term clinical outcomes[2]. Hence, there is an imperative to develop new treatments.

Mesenchymal cell activation and deposition of structural proteins, which are necessary as scaffolding for restoration of tissues disrupted by injury or infection, are evolutionarily conserved elements of almost every wound-healing response. Therefore, the therapeutic challenge is to recognize and target the molecular elements of pathogenic fibrosis to limit or reverse its progression, without globally perturbing crucial homeostatic wound-healing functions. Underlying fibrosis across organs is fibroblast activation (and expansion), typically a non-cell-autonomous phenomenon with local parenchymal and immune inputs defining its trajectory of onset and persistence. Whether fibrosis is progressive is determined by the underlying perturbation of tissue homeostasis, and in each tissue and cause of fibrosis (Fig. 1), this perturbation is distinct.

How fibrogenic signaling is initiated and why it persists are in large part related to the immune pathways activated in specific disease contexts. The causes of immune activation in fibrosis are nearly as diverse as the tissues and diseases involved, ranging from infections with viruses or mycobacteria to toxins, autoimmune disease, and aging. Although several omics approaches have been undertaken, including genome-wide association studies, metabolomics, and proteomics, single-cell RNA-sequencing (scRNA-seq) has revolutionized the capacity to interrogate the immune compartment and to find common patterns across diseases and organs. In this Review, we present an overview of the concepts arising from these advances and the immune–mesenchymal circuits suggested by the data, focusing on studies profiling human fibrotic disease for cell-type discovery and preclinical studies from which mechanistic insights have emerged.

Studying immune cell profiles in the single-cell era

The specific profiles of immune cells in human fibrotic tissues had long been elusive, but the advent of scRNA-seq approaches has already transformed our understanding of the immune landscape in tissue fibrosis. Thus, we begin with a discussion of the highlights of recent literature, focusing on transcriptomic profiling of fibrotic tissues from

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https://doi.org/10.1038/s41590-023-01551-9

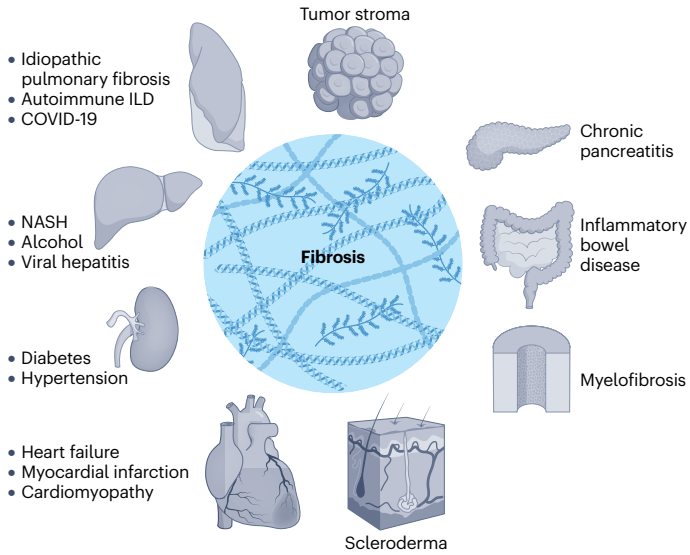

Fig. 1 | Tissular diversity of fibrosis. Fibrosis is a feature of several chronic human diseases, some of which are shown here. Immune determinants vary widely with tissular and pathogenic context. ILD, interstitial lung disease. NASH, non-alcoholic steatohepatitis.

people with key diseases. The goal is to highlight common cross-tissular themes in the immunology of pathologic fibrosis.

Liver

CLD is a huge global healthcare problem, affecting an estimated 1.5 billion people worldwide[3]. Irrespective of the cause, chronic liver damage results in fibrosis, which ultimately progresses to cirrhosis and clinical complications. The application of scRNA-seq has provided unparalleled insights into the hepatic inflammatory cell milieu in people with cirrhosis. In particular, a $CD9^+TREM2^+SPP1^+$ subpopulation of macrophages expands in individuals with cirrhosis and maps to fibrotic areas of liver tissue, and has hence been named scar-associated macrophages (SAMacs)[4,5]. By contrast, liver tissue-resident macrophages ($CD163^+MARCO^+TIMD4^+$ Kupffer cells) were not spatially localized within areas of scarring. Trajectory inference analyses have demonstrated that SAMacs are derived from the recruitment and differentiation of circulating monocytes and that the secretome of SAMacs can promote the activation of hepatic stellate cells (HSCs)[4], the main ECM-producing mesenchymal cells in fibrotic liver. A transcriptionally similar SAMac population expands in mouse models of CLD, including chronic carbon tetrachloride (CCl_4)[4] and non-alcoholic steatohepatitis (NASH)[6–10], in which they have been referred to as lipid-associated macrophages (LAMs). Lipids, such as acetylated low-density lipoprotein, can induce this macrophage phenotype in vitro[11], but SAMacs also expand in CLD in the absence of lipid accumulation.

Most of the current transcriptional profiling of human liver SAMacs has derived from individuals with advanced cirrhosis. However, SAMacs and LAMs are also present at lower numbers in healthy liver tissue and localize around areas of injury (for example, fat accumulation), even before the development of fibrosis[11]. Deconvolution analysis of bulk liver RNA-seq data[4,12] and immunostaining of liver biopsy tissue across the full spectrum of fibrosis[4,10] have confirmed that SAMac numbers expand as liver fibrosis progresses. Hence, targeting SAMacs as a therapeutic strategy may have tractability in preventing liver fibrosis progression, although direct profiling of earlier-stage fibrosis tissue is needed because the transcriptional profile of SAMacs may differ depending on disease stage or etiology.

Changes in adaptive immune cells in CLD have also been defined using scRNA-seq, with expansion of $CD4^+SELL^+CCR7^+$ T cells identified in fibrotic human liver[4]. Dedicated multiomic single-cell analysis of intrahepatic T cells has confirmed the presence of naive-like $CD4^+SELL^+CD45RA^+$ T cells in people with CLD with different etiologies, in particular primary sclerosing cholangitis, an autoimmune liver disease[13]. T cell receptor (TCR) sequencing has demonstrated that these naive-like $CD4^+$ T cells are not clonally expanded but are primed towards differentiation into an interleukin-17 (IL-17)-producing helper T cell (T_H17) effector phenotype, at least in part through epigenetic imprinting[13]. The accumulation of T_H17 cells[14] and IL-17 secretion[15] have both been associated with the promotion of liver fibrosis. Recent data have also highlighted alterations in the $CD8^+$ T cell compartment in CLD. Notably, an auto-aggressive $CXCR6^+CD8^+$ T cell population expands in human and murine NASH driven by IL-15 signaling and metabolic activation, and contributes to hepatocyte death and liver damage in a manner independent of the major histocompatibility complex[16]. $CD14^+$ myeloid cells and activated HSCs in the liver, both of which expand in fibrosis, can promote accumulation of $CD14^+CD8^+$ resident T cells, which seem to have enhanced effector and immunomodulatory functions in response to TCR ligation and Toll-like receptor 4 (TLR4) ligands[17]. It remains to be determined whether these T cell subpopulations directly regulate ECM deposition and fibrosis.

Liver scRNA-seq has also enabled the molecular definition of subpopulations of ECM-producing myofibroblasts and scar-associated endothelial cells in fibrotic human liver[4,18]. Access to specific markers of these subpopulations and spatial mapping have established the concept of the fibrotic niche, in which disease-associated cell types localize adjacent to each other and to immune cells such as SAMacs. Modeling of interactions between cells located in the fibrotic niche permits the identification of candidate pathways by which immune cells regulate fibrosis[19,20], identifying ligand–receptor pairs such as TNFSF12–TNFRSF12A, PDGFB–PDGFRA, IL-1β–IL-1R1, and AREG–EGFR between SAMacs and ECM-producing myofibroblasts[4]. Furthermore, myofibroblasts secrete ligands such as colony-stimulating factor -1 (CSF1), IL-34 and CCL2, which can regulate immune cell activation and recruitment[4]. Such analyses are providing a molecular framework for identifying candidate antifibrotic therapeutic targets.

Lung

Idiopathic pulmonary fibrosis (IPF) is the most common form of lung fibrosis, and median survival of individuals with IPF who do not receive a lung transplant is less than 4 years[21]. The etiology is poorly understood, although epithelial cell dysfunction and senescence induced by telomere attrition may represent a key predisposition that primes the lung for a fibrotic response to injury or infection[21]. The fibrotic niche in the lung is best recognized histologically by cells collecting in what are known pathologically as fibroblastic foci, areas of dense fibroblast accumulation in whorl-like structures that are associated with disease severity[22].

Recent scRNA-seq studies have characterized the immune and parenchymal cellular diversity of explanted human lungs from both deceased donors without lung disease and individuals with IPF who have received a lung transplant[23–26]. Several caveats deserve mention: the fibrotic lungs represented end-stage fibrosis in most cases; thus, inferences about immune activation at the onset of fibrosis are challenging. Second, current profiling data have in general utilized cells from dissociated whole lung rather than focusing analysis on fibroblastic foci and associated immune structures, which spatial transcriptomic techniques should address in future. Finally, IPF is predominant in the data, and other causes of lung fibrosis, such as autoimmune disease, await further study. Nonetheless, a common pattern observed across these studies was prominent fibrosis-associated change in the macrophage compartment. In healthy lungs, macrophages could be divided into at least three clusters, marked by FABP4, FCN1, or SPP1 (ref. 26). Immunofluorescence analysis has shown that all three subpopulations can be found in the lung. Interestingly, scRNA-seq analysis

of human lung lavage, which reflects the bronchial and alveolar compartments—as opposed to the interstitial spaces that are not directly continuous with the airway—revealed a predominance of FABP4$^+$ macrophages, indicating that alveolar macrophages are FABP4$^+$, similar to what has been found in mice[27]. In fibrotic samples, the same major clusters were identified in multiple studies, but with a notable change in proportion—specifically, there was a higher percentage of SPP1$^+$ macrophages, which were thought to be monocyte-derived, given their expression of genes such as *MAFB*, *CD163*, and *LGMN*, among others. This trend was supported in one study of individuals with IPF, which examined within-participant comparisons of the distribution of macrophage subtypes in fibrotic versus nonfibrotic regions of explanted IPF lung: SPP1$^+$ macrophages predominated in fibrotic regions, and FABP4$^+$ alveolar macrophages predominated in nonfibrotic regions[26]. Of note, macrophages with a profile similar that of to SPP1$^+$ macrophages in IPF were also found in proximity to fibroblasts by immunofluorescence analysis of lung samples from people who had died from COVID-19 (ref. 28); a durable lung fibrosis has been observed in people with this disease[29,30]. As mentioned for the liver, the current emphasis is on defining ligand–receptor interactions between these cells and fibroblasts or other parenchymal cells to understand the profibrotic circuits at play during fibrogenesis.

Differences in lung immune lineages besides macrophages have been more subtle in human scRNA-seq data thus far. For example, neutrophils have been shown to contribute to fibrosis in the bleomycin model[31], but more data from analysis of human fibrotic lung are needed. In comparing fibrotic and nonfibrotic regions from the same lung, a higher proportion of natural killer (NK) cells and a trend toward more B cells in nonfibrotic regions were noted[26]. A trend toward increased numbers of regulatory T cells was also observed in fibrotic IPF lung compared with healthy controls[23]. In general, the understanding of the role of lymphocytes in IPF is limited[32] and is mostly informed by preclinical models, which are context-dependent. For instance, in a chronic aspergillus infection model of lung fibrosis, profibrotic gene expression by CD44$^+$CD69$^+$CD103^{lo} tissue-resident CD4$^+$ T cells was balanced by the constraining effects of CD44$^+$CD69$^+$CD103^{hi} regulatory T cells on both inflammation and fibrosis[33]. Similarly, there is a context-specific role for type 2 immunity, such as in tissue helminthic infection[34–36]. However, the bispecific antibody SAR156597, which binds and neutralizes both IL-4 and IL-13, failed to show efficacy in a phase II placebo-controlled study of people with IPF[37]; results were similar in a phase II trial of lebrikizumab, an anti-IL-13 monoclonal antibody[38]. By contrast, in a phase 2 study of the multiorgan fibrosing disease scleroderma, SAR156597 treatment did significantly improve skin scores for people with early disease[39]. Thus, as with any chronic process, the stage of the inflammatory response and its attendant fibrotic process may be a crucial determinant of the potential efficacy of immune-focused therapies.

Kidney

Chronic kidney disease (CKD) is estimated to affect approximately 10% of the global population[40], with fibrosis being a common pathological endpoint irrespective of the etiology. As with other organs, chronic damage initially results in a fibrogenic niche, followed by fibrotic foci and ultimately widespread organ fibrosis that contributes to kidney failure[41]. Single-cell approaches have provided new insights into the cellular composition of the fibrotic kidney, initially by generating a human CKD atlas and identifying the phenotype and origin of ECM-producing myofibroblasts[42]. Notably, strong predicted ligand–receptor interactions between myeloid cells and myofibroblasts were observed, including the AREG–EGFR and PDGFB–PDGFRA pathways[42]. More detailed analysis of the myeloid cells from these CKD samples revealed a distinct SPP1$^+$APOE$^+$ macrophage subpopulation that expands in CKD, with the number of SPP1$^+$ macrophages correlating strongly with the number of collagen-expressing myofibroblasts in an independent CKD cohort[43]. Similar circulation-derived SPP1$^+$ monocyte and macrophage subpopulations with a profibrotic gene expression profile have been observed in murine models of kidney fibrosis[43,44], in which they are also predicted to have strong interactions with myofibroblasts.

scRNA-seq enables the study of rare cell populations, exemplified by the identification of basophil expansion in a murine CKD model, a finding that was validated in human CKD using scRNA-seq and CD203c immunostaining[45]. Basophil depletion attenuated fibrosis in mouse models, potentially through reduced IL-6 expression and a resultant reduction in T_H17 cell numbers[45]. Similar to the liver, renal IL-17 concentration correlated with the degree of fibrosis in human CKD[45], whereas IL-17 and T_H17 cells have both been shown to play a role in the pathogenesis of CKD-related inflammation and fibrosis[46].

Heart

Fibrosis is a common pathophysiological outcome of the majority of cardiac diseases and is associated with adverse clinical outcomes[47]. Single-cell and single-nuclear transcriptomics has been employed to study the cellular composition and transcriptional responses in healthy and diseased human heart[48]. Changes in macrophage subpopulations are well described in a variety of human cardiac diseases[49–53]. A common theme is expansion of cardiac monocyte-derived macrophages in diseased heart, with a gene expression profile including *TREM2*, *SPP1*, and *LGALS3*, alongside genes encoding inflammatory mediators, such as *IL1B*, *TNFA*, *AREG*, and *EREG*[49,52,53]. By contrast, resident cardiac macrophages (MRC1$^+$SIGLEC1$^+$CD163$^+$LYVE1$^+$) are reduced in fibrotic human heart[49,50]. Mirroring mouse fate-mapping data[54], human resident cardiac macrophages self-renew through local proliferation independent of monocyte recruitment[55] and appear to have an antifibrotic role following cardiac injury[54,56]. Pseudotime analysis has confirmed increased monocyte differentiation in the injured human heart[49], whereas reduced cardiac macrophage proliferation in the context of fibrosis[49,50,57] further supports the role of monocyte recruitment in the expansion of disease-associated macrophages. Spatial transcriptomics and in situ hybridization have been used to study human cell-type niches in the heart following myocardial infarction: disease-associated TREM2$^+$SPP1$^+$ macrophages localize adjacent to POSTN$^+$ myofibroblasts, and these macrophages are predicted to have several ligand–receptor interactions[52]. Similarly, spatial transcriptomics of fibrotic hearts maps IL-1β-expressing monocyte-derived macrophages alongside collagen-expressing FAP$^+$POSTN$^+$ fibroblasts in an immune–fibroblast niche[51], with mechanistic data in mouse models confirming that blockade of IL-1β reduces pathogenic fibroblast differentiation and fibrosis[51,58]. A population of GATA6$^+$ pericardial macrophages can infiltrate the murine heart following myocardial infarction and attenuate fibrosis; GATA6$^+$ pericardial macrophages have also been identified in human samples, but their contribution to the fibrotic niche remains unknown[59].

The human heart also contains lymphoid cells including T cells, NK cells, and B cells[60]. CD4$^+$ and CD8$^+$ T cells expand in cardiac fibrosis[50,51,53], with increased expression of activation and maturation markers, including interferon-γ (IFNγ), CCL3, CCL4, and CXCR4, in people with cardiomyopathy[50]. More refined analysis of cardiac T cells in fibrosis has been made possible by combining scRNA-seq with protein detection, assay for transposase-accessible chromatin with sequencing, and TCR sequencing, all at single-cell resolution[51,53]. Notably, cardiac T cells following acute injury have a more activated phenotype but adopt a more mature (CXCR4$^+$CD69$^+$) and exhausted (CTLA4$^+$PDCD1$^+$HAVCR2$^+$LAG3$^+$) profile in chronic fibrosis[51], suggesting that cardiac T cell function may evolve as fibrosis develops. This progressive alteration in T cell phenotype was confirmed using pseudotime, RNA velocity, and TCR clonality analysis, demonstrating that CD8$^+$ T cell clones become exhausted in chronic heart failure, whereas CD4$^+$ T cells develop a more proinflammatory and profibrotic phenotype[53]. Hence, targeting CD4$^+$ T cells could potentially be a therapeutic option. scRNA-seq

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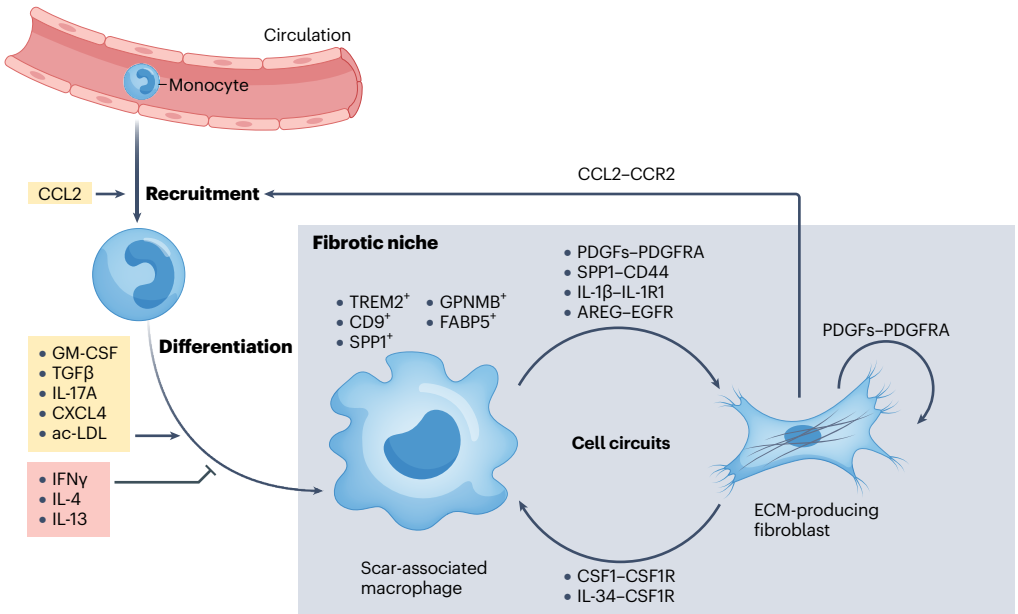

Fig. 2 | Conserved monocyte-derived SAMac phenotype within the fibrotic niche across different tissues[10]. Circulating monocytes are recruited to the tissue and differentiate into SAMacs localized within the fibrotic niche. Signals shown to promote (yellow) or inhibit (red) SAMac accumulation are displayed. Cell circuits become established between SAMacs and ECM-producing fibroblasts within the fibrotic niche, with a number of potential macrophage–fibroblast interactions. Fibroblasts can develop autocrine PDGF signaling and produce inflammatory mediators (for example, CCL2 (ref. 4)) to propagate fibrogenic pathways. ac-LDL, acetylated low-density lipoprotein.

analysis has also suggested that there is an expansion of the population of LINGO2$^+$KLRF1$^+$PRF1$^+$ NK cells in people with cardiomyopathy[57]. NK cells have previously been shown to protect against cardiac fibrosis[61], suggesting that the immune cell profile in the diseased heart may have both pro- and antifibrotic functions.

Conserved fibrosis mechanisms across organs

One of the most striking findings in the human fibrosis scRNA-seq studies is the expansion of a monocyte-derived macrophage SAMac subpopulation that expresses highly similar marker genes in different fibrotic human organs (Fig. 2). Many studies have previously demonstrated the profibrotic potential of monocyte-derived macrophages across multiple tissues[55,62–65], with ablation of these cells diminishing tissue fibrosis in injury models[27,63,66,67]. On the basis of these data, the CCR2 and CCR5 antagonist cenicriviroc has been explored in CLD clinical trials. It showed promise of antifibrotic effects in a phase II study[68], but unfortunately the phase III study was terminated early owing to lack of efficacy (ClinicalTrials.gov: NCT03028740). It should be noted that neither CCR2 nor CCR5 are specific for SAMacs, so more refined targeting approaches based on the single-cell profiles are conceptually appealing. Indeed, the ability to target SAMacs in different organs offers the opportunity to treat several fibrotic disorders.

Integrated analysis of human lung and liver scRNA-seq data has confirmed that SAMacs have a similar transcriptional profile in different organs, whereas tissue-resident macrophages (Kupffer cells and alveolar macrophages) have organ-specific profiles[10]. A combination of five markers (TREM2, CD9, SPP1, GPNMB, FABP5) could distinguish SAMacs in fibrotic liver and lung in human tissue and mouse models[10]. SAMacs depend on granulocyte–macrophage CSF, transforming growth factor-β (TGFβ), and the type 3 cytokine IL-17A for differentiation in vitro and in vivo and were able to promote pathological collagen production by liver myofibroblasts[10]. Interestingly, IL-6 from liver stromal cells seems to attenuate SAMac differentiation in vitro[5], as do other type 1 (IFNγ) and type 2 (IL-4 and IL-13) cytokines[10]. A separate comparative scRNA-seq analysis of macrophages from human kidney and cardiac fibrosis also revealed a conserved SAMac signature (SPP1, TREM2, CD9, APOE, FN1), with platelet-derived CXCL4 being crucial in the accumulation of these macrophages and promoting fibrosis in mouse models[43]. Hence, complex microenvironmental signals regulate macrophage differentiation in fibrotic organs and are likely to have a crucial role in the function of SAMacs (Fig. 2).

Another conserved theme emerging from the scRNA-seq studies of human fibrotic tissues is the concept of the fibrotic niche, in which SAMacs are topographically localized adjacent to ECM-producing fibroblasts[4,6,10,27,41,51]. The close spatial proximity of SAMac and ECM-producing fibroblasts in fibrotic foci suggests that cell–cell interactions between these populations may have a key role in regulating cellular functions (reviewed in ref. 69). Modeling of these interactions in different tissues has highlighted potentially conserved profibrotic pathways. SAMac ligands, including IL-1β, AREG, PDGF, and SPP1, have been identified, and these potentially signal to the receptors IL-1RA, EGFR, PDGFRA, and CD44 on fibroblasts[4,27,42,43,51,52,70] (Fig. 3). For interactome modeling to lead to tractable antifibrotic therapeutic targets, more functional proof of antifibrotic efficacy is needed. Data are now emerging on the antifibrotic effects of either inhibiting SAMac ligands, such as IL-1β (refs. 51,58), or attenuating SAMac accumulation in the fibrotic niche by targeting cytokines and chemokines such as IL-17A, TGFβ, or CXCL4 (refs. 10,43) in rodent models of fibrosis. However, these approaches are not specific to SAMacs and hence may have pleiotropic off-target effects on other cells and systemic immunity. Ideally, a more nuanced approach would involve specific targeting of SAMac-expressed molecules. However, it remains unknown which of these candidates represent tractable targets, with some molecules such as TREM2 having antiinflammatory and antifibrotic functions[6,71,72]. Hence, more thorough screens of the function of molecules expressed by SAMacs in fibrosis in different tissues will be essential going forward, ideally using methodologies that permit temporally controlled and cell-type-specific manipulations. Of course, the fibrotic niche also contains other cell types, such as CD4$^+$ T cells[51] and endothelial cells[4,73,74], so it is likely that more complex computational modeling approaches will be required to adequately dissect multicellular fibrogenic signaling within this niche. The broader application of spatial transcriptomic

https://doi.org/10.1038/s41590-023-01551-9

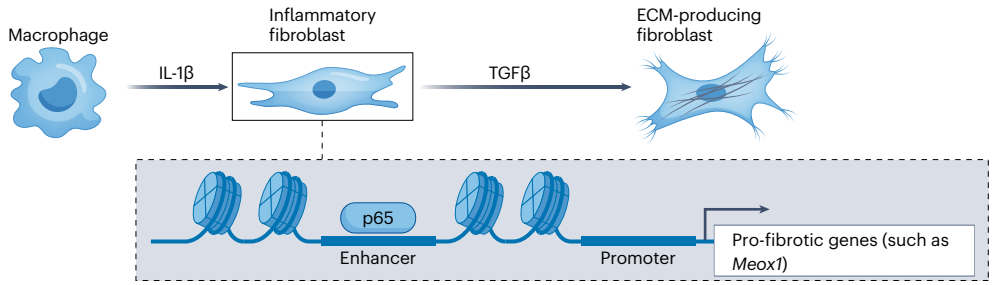

Fig. 3 | Profibrotic priming of fibroblasts by IL-1β. Using multiomic approaches in cardiac fibrosis, Alexanian et al.[58] and Lavine et al.[51] show that myeloid-cell-derived IL-1β regulates the trajectory of the fibroblast cell state towards a profibrotic profile. For instance, as shown in the figure, chromatin accessibility at an enhancer that was necessary for TGFβ-dependent induction of the profibrotic regulator gene *Meox1* was dependent on myeloid IL-1β[58]. A similar sequence of IL-1β-regulated, TGFβ-driven signaling was suggested by pseudotime analysis of fibroblasts in bleomycin-induced lung fibrosis[81].

methodologies to study fibrotic tissues[6,51,52,75,76] is likely to further inform our understanding of the cellular and molecular composition of this niche and how it might be therapeutically targeted.

Mesenchymal heterogeneity in fibrosis

In addition to detailed profiling of the immune compartment, scRNA-seq has also enhanced resolution of mesenchymal cells in disease, revealing a heterogeneity that had not been previously appreciated[42,77–80]. These studies have defined not only fibroblasts that produce high levels of ECM but also inflammatory fibroblasts with pronounced expression of chemokines and cytokines. Current work is focused on mechanistic studies using genetic tools that permit ablation of fibroblast subsets with some specificity; for example, using a novel tool, CTHRC1-CreER, to specifically ablate CTHRC1$^+$ fibroblasts that produced high levels of collagen decreased injury-induced lung fibrosis in mice[81]. In cancer, an LRRC15-DTR approach enabled ablation of mouse LRRC15$^+$ myofibroblasts, which resulted in improved T cell function and efficacy of anti-PDL1 checkpoint blockade[82].

Inflammatory fibroblasts can impact fibrogenesis directly through the elaboration of profibrotic cytokines. For example, inflammatory fibroblasts are a major source of the IL-6 family of cytokines[5,83,84] in multiple contexts in which fibrosis results from chronic inflammation, including lung fibrosis, inflammatory arthritis, inflammatory bowel disease, and CLD; indeed, the IL-6-receptor-blocking antibody tocilizumab is approved by the US Food and Drug Administration (FDA) for treatment of interstitial lung disease in the setting of systemic sclerosis. Another IL-6 family member, IL-11, is strongly induced by TGFβ in fibroblasts, and *Il-11ra1*-knockout mice were protected from fibrosis in lung, heart, and kidney models, suggestive of TGFβ-dependent profibrotic autocrine signaling[84,85]. Intriguingly, in CLD models, cytokine-expressing mesenchymal cells protect against cancer development through hepatocyte growth factor production, and ECM-producing mesenchyme promotes liver carcinogenesis through type 1 collagen production, suggesting a wider role for inflammatory fibroblasts in tissue homeostasis[80].

Similar to immune cells, the fibroblast cell state can also change dynamically, depending on microenvironmental cues. Recent findings have suggested that inflammatory fibroblasts may be primed for transition to a profibrotic, ECM-producing state. IL-1β is emerging a key player; as discussed above, Lavine et al.[51] found that IL-1β blockade or fibroblast-specific *Il1r1* deletion decreased cardiac fibrosis, and differentiation of fibroblasts into a collagen-producing state was inhibited. Similarly, in a recent lung-fibrosis study, pseudotime analysis suggested an evolutionary trajectory from inflammatory to profibrotic fibroblasts[81], and a differentiation from cytokine-expressing to ECM-expressing mesenchyme has been observed in human and mouse CLD[80]. Getting at a potential epigenetic mechanism, Alexanian et al.[58] showed that deletion of the chromatin regulator *Brd4* in CX3CR1-expressing cells (mostly monocytic lineage cells) resulted in decreased secretion of IL-1β in a murine heart-failure model. Importantly, chromatin immunoprecipitation and sequencing analysis of histone H3K27 and p65 revealed that IL-1β increased chromatin accessibility in fibroblasts of an enhancer and a p65 binding site regulating the transcription factor MEOX1, which was necessary for cardiac fibroblast activation in response to TGFβ. These studies provide evidence for a new view of fibrotic progression, accounting in part for mesenchymal heterogeneity in injured tissues and suggesting an inflammatory to profibrotic sequence in the fibroblastic cell state following interaction with macrophages[57] (Fig. 3).

Immune–fibroblast circuits in fibrosis pathogenesis

A recent study that used both mathematical modeling and empirical data from macrophage–fibroblast co-culture showed that paracrine, bidirectional signaling produced a proliferative steady state, controlling cell number[86]. This model provides a conceptual framework for understanding crosstalk in tissue fibrosis, although conditions will vary according to the dynamics of injury and repair in a multicellular environment. For instance, a single injury results in a short-lived accumulation of monocyte-derived macrophages that transiently support fibroblast accumulation, whereas repetitive or prolonged injury results in persistent accumulation of macrophages and fibroblasts leading to excess ECM deposition and a fibrotic steady state[87]. Therapeutic interruption of these bistable circuits, for example by depleting macrophages, can promote fibroblast and ECM loss if done early enough but will not impact fibrosis if performed after autocrine PDGF fibroblast circuits have been established[87] (Fig. 2).

The terms 'hot' (mainly macrophage–fibroblast circuits) and 'cold' (mainly autocrine fibroblast circuits) fibrosis have been proposed to describe these alternative fibrotic microenvironments[87]. Such hot and cold fibrotic foci can be identified in human tissue sections and can co-exist within the same diseased human kidney[88]; the principle could in the future be used to direct immunomodulatory therapeutics to patients with hot fibrosis, in whom antifibrotic drugs are more likely to be effective. Of course, the complexity of signals that regulate macrophage–fibroblast circuits are only starting to be unraveled: multiple predicted ligand–receptor pairs have been identified from scRNA-seq studies, and other cell types are also found within the fibrotic niche (as discussed above)[77,78]. For example, in one cross-tissue meta-analysis, correlations were found between immune cell proportions and the proportions of two specific fibroblast subsets labeled inflammatory fibroblasts: CXCL10$^+$CCL19$^+$ and SPARC$^+$COL3A1$^+$ cells[89]. Ligand–receptor analysis predicted interaction between the CXCL10$^+$CCL19$^+$ cells and T cells, whereas the SPARC$^+$COL3A1$^+$ cells were predicted to interact with the vascular endothelium. These predictions were confirmed with marker visualization by microscopy,

https://doi.org/10.1038/s41590-023-01551-9

co-culture experiments, and comparison with in vivo models. In the coming years, an expanded map of interactions among multiple cell types in fibrosis will require more complex mathematical models incorporating multidirectional signaling to model the fibrotic niche, and ideally in silico predictions of responses to candidate therapeutic interventions will be made[5,83,90].

Contact-dependent interactions between fibroblasts and macrophages, for example via cadherin-11, are also crucial to functional signaling[91]. Thus, both the fibroblast secretome and surface protein expression may regulate immune cell accumulation and activation in fibrotic disorders. In a similar vein, the capacity of fibroblasts to present antigens to T cells in the absence of specialized antigen-presenting cells has long been suggested[92,93]. This ability has mostly been attributed to a subset of fibroblasts in cancer that can promote $CD4^+$ T cell activation[94,95]. The functional role of fibroblast antigen presentation has also recently been described in mouse cardiac fibrosis models, in which IFNγ production induces major histocompatibility complex class II expression by cardiac fibroblasts, resulting in $CD4^+$ T cell activation promoting fibrosis[96]. It remains to be determined whether similar mechanisms exist in human fibrotic diseases.

ECM modulation of immune cell function

The ECM itself can regulate a range of immune cell functions via its molecular, physical, and mechanical properties (reviewed in detail in ref. 97). Fibrotic ECM shows alterations in not only fibrillar molecules such as collagens, but also glucosaminoglycans and proteoglycans. These ECM components can directly signal to immune cells; for example, hyaluronan binds to CD44 to promote leukocyte or platelet retention in diseased tissue[97–99]. The ECM also contains a range of growth factors, cytokines, and chemokines that have crucial immunomodulatory roles (reviewed in ref. 100). The deposition of excess fibrillar collagen in fibrotic disorders results in reduced mechanical compliance and increased tissue stiffness. Immune cell function is also regulated through stiffness-sensing mechanisms; for example, monocyte-derived macrophages sensing cyclical stretch in the lung activated a HIF1-α-dependent inflammatory program dependent on the mechanosensitive Piezo1 calcium channel in the plasma membrane that was necessary for fibrosis[101]. Macrophage metabolic pathways regulating collagen synthesis are also mechanosensitive. In the context of tumor-associated fibrosis, both TGFβ and stiffness induced a macrophage collagen-biosynthesis pathway and ARG1 expression, with the data suggesting that ARG1-dependent arginine to proline flux regulates collagen synthesis[102]. Notably, mechanical communication by fibroblast contraction-induced deformation fields in ECM can be sensed by macrophages through $\alpha_2\beta_1$ integrin binding and stretch-sensitive ion channels, resulting in the migration of the macrophages towards fibroblasts[103]. Modulation of the ECM sensing mechanisms that can regulate immune cells may therefore be a novel therapeutic avenue to disrupt macrophage–fibroblast circuits.

Immune regulation of fibrosis regression

Fibrosis is a highly dynamic process, with the potential for ECM remodeling, fibrosis regression, and the restoration of tissue architecture now well described in clinical studies of CLD[104,105], cardiac fibrosis[106–108], and CKD[109,110]. Preclinical studies have shown that the immune system plays a key role in fibrosis regression (Fig. 4). Macrophages have been the most widely studied, with depletion of macrophages during murine liver and lung fibrosis regression resulting in a failure of ECM remodeling and persistent scarring[66,111,112]. These restorative macrophage functions are mediated by $TLR4^+LY6C^{lo}$Stabilin-1^+ monocyte-derived macrophages[111,113,114], following a phenotypic switch[111]. During fibrosis regression, macrophages downregulate proinflammatory cytokine and chemokine expression and upregulate the expression of matrix metalloproteinase (MMP) enzymes[111], including MMP-12 and MMP-13, which can promote degradation of fibrotic ECM[115–118]. Macrophages are also capable of directly internalizing collagen fragments via ApoE[119] or MFGE8 binding[120] and uptake for lysosomal degradation[121].

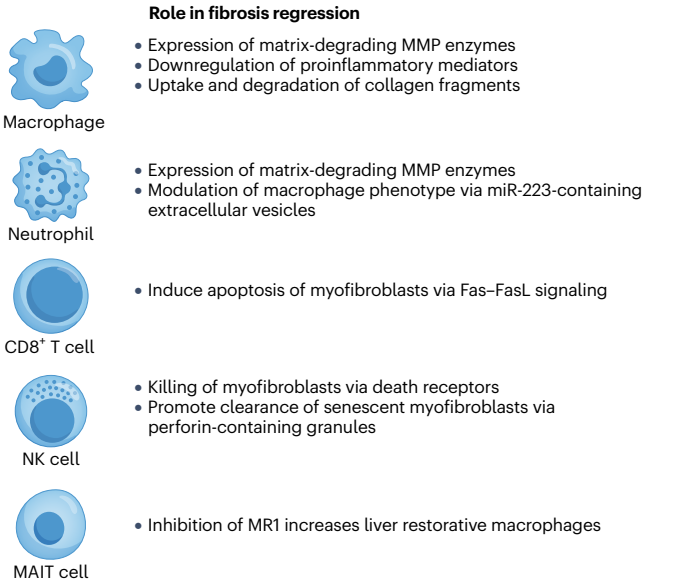
Role in fibrosis regression
Macrophage
• Expression of matrix-degrading MMP enzymes
• Downregulation of proinflammatory mediators
• Uptake and degradation of collagen fragments
Neutrophil
• Expression of matrix-degrading MMP enzymes
• Modulation of macrophage phenotype via miR-223-containing extracellular vesicles
CD8+ T cell
• Induce apoptosis of myofibroblasts via Fas–FasL signaling
NK cell
• Killing of myofibroblasts via death receptors
• Promote clearance of senescent myofibroblasts via perforin-containing granules
MAIT cell
• Inhibition of MR1 increases liver restorative macrophages

Fig. 4 | Fibrosis regression. Summary of the role of immune cell types in regulating the resolution of fibrosis.

Factors that regulate the switch in liver macrophage phenotype from profibrotic to restorative include phagocytosis[111], STAT3–IL-10–IL-6 signaling[122], activity of the transcription factor NUR77 (ref. 123), and microbiome-driven TLR4 and FXR agonism[114]. Cell-to-cell interactions with other immune cells within the hepatic fibrotic niche also regulate macrophage phenotypic conversion. Neutrophils, for example, can promote fibrosis regression in mouse CLD models by expression of MMPs[124] or signaling to macrophages through extracellular vesicles containing the microRNA miR-223, resulting in macrophage NLRP3 silencing and reprogramming into a restorative phenotype[125]. Mucosal-associated invariant T (MAIT) cells, non-conventional T cells, accumulate in the liver fibrotic niche and promote fibrosis progression[126], whereas blockade of MAIT cell activation using acetyl-6-formylpterin accelerated fibrosis regression through increased numbers of $Ly6C^{lo}$ restorative macrophages[127]. Other therapeutic approaches that modulate macrophage phenotype in vivo to promote liver fibrosis regression include liposome administration[111], Spiegelmer-based inhibition of CCL2 (ref. 128), and nanoparticles promoting hepatic myofibroblast relaxin production, leading to increased miR-30a-5p-containing exosome signaling to macrophages to enhance pro-resolution functions[123]. These data demonstrate the potential to therapeutically target immune cells in an established fibrotic niche in vivo to promote fibrosis regression. However, an understanding of the pro-resolution macrophage phenotype from human samples remains lacking in the liver and other tissues and is essential to enable effective translation from preclinical findings.

In addition to ECM degradation, the loss of ECM-producing mesenchymal cells is crucial for effective fibrosis regression. In the liver, following the cessation of injury, activated HSCs have been shown to undergo apoptosis, become senescent, or to become deactivated and downregulate collagen gene expression (reviewed in ref. 129). NK cells have a role in killing activated HSCs via binding to NKG2D receptors or activation of death receptors, such as TRAIL[129,130]. Recent data also suggest that a subpopulation of IL-15-dependent $CD8^+$ tissue-resident memory T cells can induce apoptosis of activated HSCs via Fas–FasL signaling in murine NASH[131]. Senescent HSCs, which are less fibrogenic than non-senescent counterparts, were also cleared by NK cells resulting in more rapid resolution of fibrosis[132]; this effect appears to be

dependent on exocytosis of perforin-containing granules[133]. Finally, activated HSCs can revert to a quiescent phenotype with downregulation of ECM expression[134,135], but remain primed for more intense reactivation in response to repeated injury[135]. However, the signals regulating deactivation and the role of immune cells in this process remain incompletely understood. Indeed, the fate of myofibroblasts and the relative contribution of cell death, senescence, and deactivation in human fibrosis regression are currently unknown.

Factors regulating inflammatory responses in fibrosis

We have focused this review on immune cell heterogeneity and function in human tissue fibrosis, but a number of additional factors have also been associated with fibrosis pathogenesis, and their impact on immunobiology is not yet known. Genomic analyses, for example, have been performed in people with fibrosis in different organs, identifying a range of genetic susceptibility loci, such as *PNPLA3* and *HSD17B13* in liver fibrosis[136,137]; *MUC5B* and *TERT* in lung fibrosis[138]; and *SLC2A12* and *HFE* in cardiac fibrosis[139]. In contrast to the conserved inflammatory and mesenchymal responses discussed above, there is little overlap in the genes that confer susceptibility to fibrosis in different organs, suggesting that the aberrant epithelial injury responses that initiate fibrosis are likely to be tissue-specific. How these differing epithelial responses lead to a conserved inflammatory and profibrotic program needs to be integrated into models of fibrosis pathogenesis in the future. Aging is also associated with increased rates of fibrosis in different organs[140–142], with processes such as telomere shortening, senescence and mitochondrial dysfunction thought to impair tissue regeneration and enhance pathological fibrosis (reviewed in ref. 140). Aging can also modulate inflammatory responses, a process termed inflammaging, which is characterized by low-grade chronic inflammation[143,144] and altered functional responses of innate and adaptive immune cells[145–147]. However, the role of inflammaging in tissue fibrosis remains understudied and is important to consider when studying profibrotic mechanisms and identifying therapeutic targets in cohorts of individuals with disease. In the future, omics profiling and immune phenotyping studies in human fibrotic disorders should incorporate a range of clinical metadata, such as age, sex, ethnicity, genetics, disease stage, and ideally long-term clinical outcomes, to properly understand how other factors influence inflammatory and profibrotic circuits.

Multiomic profiling of fibrotic tissue

Other omics approaches have also been used in studies of people with fibrotic disorders. High-dimensional proteomics in the circulation has identified candidate biomarkers of liver[148–150] and pulmonary fibrosis[151], including inflammation-associated molecules such as TREM2 and IL-18R1 (refs. 148,149), whereas metabolomic analysis has defined metabolites that are associated with tissue fibrosis[152,153]. However, the mechanistic links between these systemic changes and specific immune cell phenotypes within the fibrotic niche often remain unclear. Advances in technology, specifically the ability to perform such analyses at the single-cell level, will address this issue in forthcoming years. Crucially, the development of spatially resolved omics approaches, which can be applied to fresh or archival tissue samples without tissue dissociation, is allowing study of cell–cell circuits in the fibrotic niche in situ for the first time[6,51,52,75,76]. As the resolution and depth of these methodologies improve, they promise to herald the next wave of therapeutic target discovery in tissue fibrosis.

Improving fibrosis models

The complex, multicellular nature of fibrosis necessitates the use of well-phenotyped and reproducible models to study candidate therapeutic interventions. Traditionally, researchers have largely relied on rodent models, but the fact that these models often develop fibrosis over weeks or months, rather than years or decades, as in people, and the poor translational success rate between rodent targets and effective human therapies highlight the need for better models, using human cells and tissues where possible. Several such methodologies have now been established, including organoids and spheroids, organ-on-a-chip systems, and precision-cut tissue slices (reviewed in ref. 154), which are becoming widely used to study disease mechanisms, understanding cell–cell interactions, and performing drug screens. However, many of these models have not yet incorporated relevant immune cells, lacking the functional blood supply that is needed for immune cell recruitment, differentiation, and turnover. In vivo fibrosis models using humanized mice may have a role in addressing this concern but are often performed in an immunocompromised background strain. Further refinements of such in vivo and in vitro models, supplemented with detailed molecular profiling at single-cell resolution, are therefore urgently needed to determine which models best recapitulate immune cell phenotypes and functions in fibrosis. Going forwards, it is also crucial that target identification and model refinement are informed by profiling samples from individuals with disease, and cells, such as SAMacs, from earlier-stage fibrotic disease rather than the advanced disease stages that are currently over-represented in many single-cell studies. These data will progress our understanding of the key disease stages at which immune inputs regulate fibrogenesis and hence can be targeted therapeutically.

Conclusions and outlook

In this Review, we have summarized recent exciting work mapping the immune identities of cells in fibrotic tissues. With the unprecedented resolution of single-cell analysis, this literature has provided a view of the heterogeneity of cell types while highlighting certain recurrent themes, including the transcriptomic profile of profibrotic SAMacs and the importance of paracrine signaling between immune and mesenchymal cells. Future work should incorporate spatial information at a single-cell, genome-wide scale across the full disease spectrum and will be enhanced by multiomic mapping, including proteomic, metabolomic, and epigenomic data. Ultimately, we anticipate these findings will align fibrotic diseases with cancer, where targeted immunomodulatory treatments are a realistic and effective therapeutic option.

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Acknowledgements

M.B. is supported by a US DOD CDMRP Investigator-Initiated Research Award (W81XWH2110417). P.R. is supported by a Medical Research Council Senior Clinical Fellowship (MR/W015919/1). Images were made with copyright (M.B.) using Biorender.

Author contributions

M.B. and P.R. conceived and co-wrote the manuscript, with equal contribution.

Competing interests

M.B has performed consultancy work for Merck and Ono Pharma. P.R. has received research support from Genentech, Intercept Pharmaceuticals, and NeoGenomics. P.R. has performed consultancy work for Merck.

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Peer review information *Nature Immunology* thanks Thomas Wynn and Matthew Buechler for their contribution to the peer review of this work. Jamie D. K. Wilson, in collaboration with the *Nature Immunology* team.

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